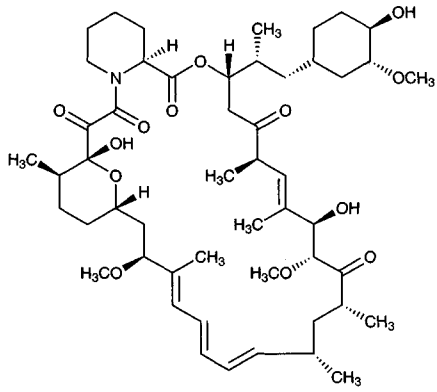
Injection volume: 20
Detector: E, BAS LC-4, TL-5 glassy carbon electrode +1.0 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 5
Limit of quantitation: 0.2 ng

KEY WORDS
rat; brain

REFERENCE
Sauter,A.; Frick,W. Determination of cholecystokinin tetrapeptide and cholecystokinin octapeptide sulfate in different rat brain regions by high-pressure liquid chromatography with electrochemical detection, *Anal.Biochem.*, **1983**, *133*, 307–313.

Sirolimus



Molecular formula: $C_{51}H_{79}NO_{13}$
Molecular weight: 914.19
CAS Registry No.: 53123-88-9
Merck Index: 8288

SAMPLE
Matrix: blood, tissue
Sample preparation: Vortex 0.05-1 mL whole blood or hepatic microsomes with 1 μg IS and 1 mL 100 mM sodium carbonate for 10 s. Add 10 mL MTBE, shake horizontally for 30 min. Centrifuge at 1500 g for 10 min at 4°, remove the organic layer, evaporate to dryness under a stream of nitrogen. Reconstitute the residue in 200 μL mobile phase. Centrifuge at 2500 g at 4°. Inject a 150 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2 mm 37-53 μm C18 pellicular (Upchurch)
Column: 250 × 4.6 5 μm Supelco LC-318 C18
Mobile phase: MeOH:water 70:30
Column temperature: 45
Flow rate: 1.0
Injection volume: 150
Detector: UV 278

CHROMATOGRAM
Retention time: 22, 32 (isomers)
Internal standard: N-undecyl-o-toluamide (Prepare by dispersing N-undecylamine in cold NaOH and adding an equimolar amount of o-toluoyl chloride, shake vigorously. Remove the product by filtration, wash, air dry, recrystallize from EtOH/water.) (27.5)
Limit of detection: 1 ng
Limit of quantitation: 2.5 ng

OTHER SUBSTANCES
Simultaneous: beclomethasone, corticosterone, cyclosporine A and G, erythromycin, ethinyl estradiol, hydrocortisone, ketoconazole, lorazepam, methylprednisolone, norethindrone, prednisolone, prednisone, propranolol, rifampicin, tacrolimus

KEY WORDS
rat; rabbit; human; whole blood; microsomes

REFERENCE
Ferron,G.M.; Conway,W.D.; Jusko,W.J. Lipophilic benzamide and anilide derivatives as high-performance liquid chromatography internal standards: application to sirolimus (rapamycin) determination, *J.Chromatogr.B*, **1997**, *703*, 243–251.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Condition a 3 mL C18 SPE cartridge (LiChroprep, Merck) with 3 mL MeCN and 3 mL pH 3.0 sulfuric acid. Add 500 μL MeCN to 1.5 mL microsomal incubation. Centrifuge at 2500 g for 2 min. Add the supernatant to the SPE cartridge, wash with 3 mL MeOH:pH 3.0 sulfuric acid 50:50 and 500 μL hexane. Dry cartridge by drawing air through it and elute with 1.5 mL dichloromethane. Evaporate the eluate under a stream of nitrogen at 40°. Reconstitute the residue in 300 μL MeCN:pH 3.0 sulfuric acid 75:25, wash with 500 :gmL hexane. Inject a 125 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 3 μm Hypersil C8
Mobile phase: Gradient. A was MeCN. B was pH 3.0 sulfuric acid. A:B maintain at 47:53 for 7 min, to 50:50 over 13 min, to 55:45 over 20 min, to 61:39 over 5 min, wash with 95:5 for 5 min, re-equilibrate at initial conditions for 7 min.
Column temperature: 40
Flow rate: 0.7
Injection volume: 125
Detector: UV 276

CHROMATOGRAM
Retention time: 33
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: acetaminophen, aspirin, amphotericin B, bromocryptine, captopril, corticosterone, cyclosporine, dexamethasone, diclofenac, erythromycin, ergotamine tartrate, diethyldithiocarbamate, disulfiram, ethinyl estradiol, josamycin, ketoconazole, lidocaine, methylprednisolone, miconazole, α-naphthoflavone, naringin, nifedipine, omeprazole, phenytoin, prednisolone, progesterone, propranolol, quinidine, ranitidine, sulfaphenazole, trimethoprim, troleandomycin, verapamil

KEY WORDS
rat; pharmacokinetics; human; pig; small intestine; liver; SPE

REFERENCE
Lampen,A.; Zhang,Y.; Hackbarth,I.; Benet,L.Z.; Sewing,K.-F.; Christians,U. Metabolism and transport of the macrolide immunosuppressant sirolimus in the small intestine, *J.Pharmacol.Exp.Ther.*, **1998**, *285*, 1104–1112.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Condition a 3 mL C8 SPE cartridge (Recipe, Germany) with 3 mL MeCN and 1.5 mL water. Mix 1.5 mL microsomal incubation with 2 mL MeOH:saturated zinc sulfate in water 50:50, add 10 μL 1 mM IS in MeCN:pH 3.0 sulfuric acid 75:25. Centrifuge at 2500 g. Add the supernatant to the SPE cartridge, wash with 3 mL water. Dry cartridge by drawing air through it for 3 min, elute with 400 μL MeCN:0.1% formic acid 90:10 by centrifuging at 800 g for 2 min. Inject a 25 μL aliquot of the extract.

HPLC VARIABLES
Column: 250 × 4 3 μm Hypersil C8
Mobile phase: MeOH:0.1% formic acid 90:10

Column temperature: 35
Flow rate: 0.5
Injection volume: 25
Detector: MS, HP 5989A, ESI 59887A, drying gas 350°, quadrupole 120°, capillary exit voltage 200 V, positive ion mode, multiplier voltage 2740 V, X-ray 10000 V, m/z 936

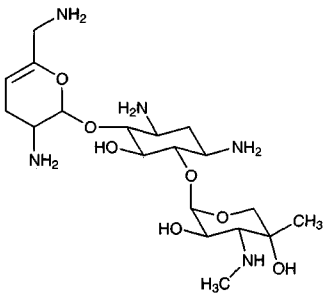
CHROMATOGRAM
Limit of detection: 250 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; pharmacokinetics; human; pig; small intestine; liver; SPE

REFERENCE
Lampen,A.; Zhang,Y.; Hackbarth,I.; Benet,L.Z.; Sewing,K.-F.; Christians,U. Metabolism and transport of the macrolide immunosuppressant sirolimus in the small intestine, *J.Pharmacol.Exp.Ther.*, **1998**, *285*, 1104–1112.

Sisomicin



Molecular formula: $C_{19}H_{37}N_5O_7$
Molecular weight: 447.53
CAS Registry No.: 32385-11-8, 53179-09-2 (sulfate)
Merck Index: 8695

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

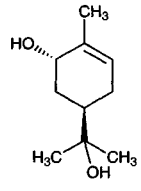
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 14.032

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Sobrerol



Molecular formula: $C_{10}H_{18}O_2$
Molecular weight: 170.25
CAS Registry No.: 498-71-5
Merck Index: 8707

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb RP18
Mobile phase: EtOH:water 20:80 with 10 mM β-cyclodextrin and 0.5 mM tri-O-methyl-β-cyclodextrin
Column temperature: 25
Flow rate: 0.04
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: k' 3.4

OTHER SUBSTANCES
Extracted: morsuximide, mephenytoin

KEY WORDS
no chiral separation

REFERENCE
Nowakowski,R.; Bielejewska,A.; Duszczyk,K.; Sybilska,D. Chiral discrimination by high-performance liquid chromatography with joint use of two cyclodextrin additives, *J.Chromatogr.A*, **1997**, *782*, 1–11.

Sodium nitroprusside

$Na_2[Fe(CN)_5NO]$

Molecular formula: $C_5FeN_6Na_2O$
Molecular weight: 261.92
CAS Registry No.: 14402-89-2, 13755-38-9 (dihydrate)
Merck Index: 8794

SAMPLE
Matrix: blood
Sample preparation: 100 μL Whole blood or plasma + 100 μL 3 M perchloric acid, mix, let stand at 0° for 5 min, centrifuge at 8000 g for 5 min. Remove a 150 μL aliquot of the supernatant and add it to 250 μL 1 M K_2HPO_4, mix vigorously, centrifuge for 8000 g for 5 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4 Asahipak BEST 502Q
Mobile phase: 100 mM pH 6.0 Acetate buffer containing 300 mM sodium perchlorate
Flow rate: 0.5
Injection volume: 20
Detector: F ex 583 em 607 following post-column reaction. The column effluent mixed with 5 mM dithiothreitol in 50 mM pH 9.0 Tris-HCl buffer containing 5 mM EDTA pumped at 0.1 mL/min and this mixture flowed through a 4.5 m × 0.5 mm ID coil at 90°. The effluent from this coil mixed with 0.5% Chloramine T in water pumped at 0.1 mL/min and flowed through a mixing coil. The effluent from this coil mixed with reagent pumped at 0.1 mL/min and this mixture flowed through a mixing coil to the detector. (Reagent was a mixture of 1.5 g barbituric acid, 15 mL pyridine, 3 mL concentrated HCl, and 82 mL water. Nitroprusside is reduced to cyanide with dithiothreitol. The cyanide is converted to cyanogen chloride with chloramine T, the cyanogen chloride reacts with the pyridine to form pent-2-en-1,5-dial, and this compound reacts with barbituric acid to form the fluorescent 5,5'-(1,3-pentadiene-1-yl-5-ylidene) dibarbituric acid.)

CHROMATOGRAM
Retention time: 17
Limit of detection: 200 fmole

OTHER SUBSTANCES
Noninterfering: cyanide, thiocyanate

KEY WORDS
post-column reaction; whole blood; plasma; rat; pharmacokinetics

REFERENCE
Watanabe,T.; Nagamine,Y.; Toida,T.; Koshiishi,I.; Imanari,T. Sensitive determination of nitroprusside in blood by high performance liquid chromatography, *Anal.Sci.*, **1988**, *4*, 203–206.

SAMPLE
Matrix: formulations
Sample preparation: Inject an aliquot of the solution.

HPLC VARIABLES
Column: 300 × 3.9 10 μm phenyl (Waters)
Mobile phase: MeCN:buffer 30:70 (Buffer was 10 mM KH_2PO_4 and 5 mM tetrabutylammonium hydroxide adjusted to pH 7.1 with phosphoric acid.)
Flow rate: 2
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 6
Limit of quantitation: 10 μg/mL

OTHER SUBSTANCES
Simultaneous: degradation products, ferricyanide, ferrocyanide

KEY WORDS
stability-indicating; injections; 5% dextrose

REFERENCE
Baaske,D.M.; Smith,M.D.; Karnatz,N.; Carter,J.E. High-performance liquid chromatographic determination of sodium nitroprusside, *J.Chromatogr.*, **1981**, *212*, 339–346.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: Partisil 10-SAX
Mobile phase: 500 mM KH_2PO_4 adjusted to pH 3.0 with phosphoric acid
Flow rate: 1.4
Injection volume: 50
Detector: UV 230

CHROMATOGRAM
Retention time: 5

KEY WORDS
injections; 5% dextrose; saline; lactated Ringer's solution

REFERENCE
Mahony,C.; Brown,J.E.; Stargel,W.W.; Verghese,C.P.; Bjornsson,T.D. In vitro stability of sodium nitroprusside solutions for intravenous administration, *J.Pharm.Sci.*, **1984**, *73*, 838–839.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: phenyl
Mobile phase: MeCN:buffer 30:70 (Buffer was 10 mM KH_2PO_4 containing 0.52% tetrabutylammonium phosphate, pH adjusted to 7.1 with KOH.)
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 7.2
Internal standard: salicylic acid (6)

KEY WORDS
injections; 5% dextrose; stability-indicating

REFERENCE
Pramar,Y.; Das Gupta,V.; Gardner,S.N.; Yau,B. Stabilities of dobutamine, dopamine, nitroglycerin and sodium nitroprusside in disposable plastic syringes, *J.Clin.Pharm.Ther.*, **1991**, *16*, 203–207.

Somatomedin

Molecular weight: 7649
CAS Registry No.: 67763-96-6
Merck Index: 8862

SAMPLE
Matrix: blood
Sample preparation: Prepare a 240:10 serum:concentrated formic acid mixture, let stand for 1 h at room temperature or 37°, filter (0.22 μm) or centrifuge at 12000 g for 5 min, inject a 200 μL aliquot of the filtrate or supernatant.

HPLC VARIABLES
Guard column: 100 × 7.5 10 μm TSK 2000 SW (Toyo Soda)
Column: 600 × 7.5 10 μm TSK 2000 SW (Toyo Soda)
Mobile phase: 100 mM Ammonium formate adjusted to pH 3.0 with concentrated formic acid
Flow rate: 0.7
Injection volume: 200
Detector: UV 280 or bioassay

CHROMATOGRAM
Retention time: 14

KEY WORDS
serum; SEC; human; rat

REFERENCE
Goldstein,S.; Stivaletta,L.A.; Phillips,L.S. Separation of somatomedins and somatomedin inhibitors by size exclusion high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *339*, 388–393.

SAMPLE
Matrix: cell suspensions
Sample preparation: Centrifuge cells in modified Eagle's medium containing 10% fetal bovine serum, 20 mM L-glutamine, 50 U/mL penicillin, and 50 μg/mL streptomycin at 2500 g, to each 1 liter of supernatant add 390 g ammonium sulfate, dissolve, let stand overnight, centrifuge at 4° at 10000 g for 30 min. Dissolve the precipitate in 25-30 mL 50 mM pH 7.8 ammonium bicarbonate, purify 40-50 mL aliquots on a 1000 × 50 column of Sephacryl S-300, elute with 50 mM pH 7.8 ammonium bicarbonate buffer at 0.7 mL/min at 4°, adjust pH of eluate to 6.5 with acetic acid, inject a 10-15 mL aliquot at 0.5 mL/min.

HPLC VARIABLES
Column: 250 × 4.6 7 μm Aquapore RP-300 C8 (Brownlee)
Mobile phase: Gradient. MeCN:buffer from 0:100 to 20:80 over 10 min, to 60:40 over 75 min. (Buffer was 100 mM pH 6.5 ammonium acetate, pass through a C18 Sep-Pak to remove impurities.)
Flow rate: 0.5 (?)
Injection volume: 10000-15000
Detector: UV 280

CHROMATOGRAM
Retention time: 40

REFERENCE
Powell,D.R.; Lee,P.D.K.; Shively,J.E.; Eckenhausen,M.; Hintz,R.L. Method for purification of an insulin-like growth factor -binding protein produced by human HEP G2 hepatoma cells, *J.Chromatogr.*, **1987**, *420*, 163–170.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 75 × 4.6 Beckman RPSC
Mobile phase: Gradient. MeCN:10 mM trifluoroacetic acid from 10:90 to 60:40 over 40 min
Flow rate: 1
Detector: UV 210

CHROMATOGRAM
Retention time: 33

REFERENCE
Saito,Y.; Yamada,H.; Niwa,M.; Ueda,I. Production and isolation of recombinant somatomedin C, *J.Biochem.(Tokyo)*, **1987**, *101*, 123–134.

SAMPLE
Matrix: solutions
Sample preparation: Adjust pH to 3.0 with trifluoroacetic acid (if necessary), filter (0.22 μm) (if necessary), inject a 200 μL aliquot.

HPLC VARIABLES
Column: 100 × 3.9 Aquapore RP-300 (Brownlee)

Mobile phase: Gradient. A was water containing 0.1% trifluoroacetic acid. B was MeCN:water 80:20 containing 0.1% trifluoroacetic acid. A:B from 30:70 to 50:50 over 20 min, maintain at 50:50 for 3 min, to 0:100 over 3 min, return to initial conditions over 2 min.
Flow rate: 1
Injection volume: 200
Detector: UV 280

CHROMATOGRAM
Retention time: 12 (isoform 1), 14 (isoform 2), 2 (reduced form)

REFERENCE
Meng,H.; Burleigh,B.D.; Kelly,G.M. Reduction studies on bacterial recombinant somatomedin C/insulin-like growth factor-1, *J.Chromatogr.*, **1988**, *443*, 183–192.

Somatrem

Molecular formula: $C_{995}H_{1537}N_{263}O_{301}S_8$
Molecular weight: 22256.39
CAS Registry No.: 82030-87-3
Merck Index: 8864

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm C4 (Vydac)
Mobile phase: Isopropanol:500 mM pH 6.5 KH_2PO_4 29:71
Column temperature: 45
Flow rate: 1
Detector: F ex 295 em 348

CHROMATOGRAM
Retention time: 25

OTHER SUBSTANCES
Simultaneous: somatropin

REFERENCE
Oroszlan,P.; Wicar,S.; Teshima,G.; Wu,S.L.; Hancock,W.S.; Karger,B.L. Conformational effects in the reversed-phase chromatographic behavior of recombinant human growth hormone (rhGH) and N-methionyl recombinant human growth hormone (Met-hGH), *Anal.Chem.*, **1992**, *64*, 1623–1631.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Vydac C4 Protein Pak
Mobile phase: Gradient. MeCN:0.05% trifluoroacetic acid from 43:57 to 55:45 over 30 min
Column temperature: 45
Flow rate: 0.5
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 35

OTHER SUBSTANCES
Simultaneous: somatropin

REFERENCE
Arcelloni,C.; Fermo,I.; Banfi,G.; Pontiroli,A.E.; Paroni,R. Capillary electrophoresis for protein analysis: separation of human growth hormone and human insulin molecular forms, *Anal.Biochem.*, **1993**, *212*, 160–167.

Somatropin

Molecular formula: $C_{990}H_{1529}N_{263}O_{299}S_7$
Molecular weight: 22124.21
CAS Registry No.: 9002-72-6, 12629-01-5 (human)
Merck Index: 8864

SAMPLE
Matrix: bulk
Sample preparation: Prepare a solution in 20 mM sodium borate containing 1.44 mM EDTA adjusted to pH 9.5 with NaOH, inject an aliquot.

HPLC VARIABLES
Column: Vydac 218TP104
Mobile phase: Gradient. A was MeCN:0.1% trifluoroacetic acid:water 10.5:70:19.5. B was MeCN:0.1% trifluoroacetic acid:water 21:70:9. A:B from 100:0 to 0:100 in 30 min
Flow rate: 1
Injection volume: 20
Detector: UV 214

CHROMATOGRAM
Retention time: 22.5 (monomer), 25 (dimer)

REFERENCE
Chang,J.P.; Ferguson,T.H.; Record,P.A.; Dickson,D.A.; Kiehl,E.; Kennington,A.S. Improved potency assay for recombinant bovine somatotropin by high-performance size-exclusion chromatography, *J.Chromatogr.A*, **1996**, *736*, 97–104.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a solution in 20 mM sodium borate containing 1.44 mM EDTA adjusted to pH 9.5 with NaOH, inject an aliquot.

HPLC VARIABLES
Column: 300 × 21.5 TSK G3000SW
Mobile phase: 20 mM Sodium borate containing 1.44 mM EDTA, adjusted to pH 7.3 with HCl
Flow rate: 0.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 13.3 (dimer), 14.5 (monomer)

REFERENCE
Chang,J.P.; Ferguson,T.H.; Record,P.A.; Dickson,D.A.; Kiehl,E.; Kennington,A.S. Improved potency assay for recombinant bovine somatotropin by high-performance size-exclusion chromatography, *J.Chromatogr.A*, **1996**, *736*, 97–104.

SAMPLE
Matrix: fermentation broth

Sample preparation: Centrifuge 1.5 mL fermentation broth at 16000 g for 2 min, put the pellet on ice. Re-suspend the pellet in 150 μL ice-cold 10 mM pH 7.5 Tris-HCl containing 20% (w/v) sucrose. Add 5 μL 500 mM pH 8.0 EDTA, incubate on ice for 10 min. Microcentrifuge the cells and re-suspend the pellets in 100 μL cold 1 mM pH 7.5 Tris-HCl solution. Incubate the mixture for 10 min on ice and centrifuge again for 5 min. Remove the supernatant and inject an aliquot.

HPLC VARIABLES
Guard column: Vydac 214 FSK 54
Column: 250 × 4.6 5 μm Vydac 214 TP 54 C4
Mobile phase: n-Propanol:50 mM pH 7.5 Tris-hydrochloric acid buffer 29:71 (Place a column of 7.9-12.4 μm LiChrosorb Si 60 between the pump and injector.)
Column temperature: 45
Flow rate: 0.5
Injection volume: 40-70
Detector: UV 220

CHROMATOGRAM
Retention time: 29

OTHER SUBSTANCES
Extracted: degradation products

KEY WORDS
comparison with SEC

REFERENCE
Dalmora,S.; Ezequiel de Oliveira,J.; Affonso,R.; Gimbo,E.; Ribela,M.T.C.P.; Bartolini,P. Analysis of recombinant human growth hormone directly in osmotic shock fluids, *J.Chromatogr.A*, **1997**, *782*, 199–210.

SAMPLE
Matrix: fermentation broth
Sample preparation: Centrifuge 1.5 mL fermentation broth at 16 000 g for 2 min, put the pellet on ice. Re-suspend the pellet in 150 μL ice-cold 10 mM pH 7.5 Tris-HCl containing 20% (w/v) sucrose. Add 5 μL 500 mM pH 8.0 EDTA, incubate on ice for 10 min. Microcentrifuge the cells and re-suspend the pellets in 100 μL cold 1 mM pH 7.5 Tris-HCl solution. Incubate the mixture for 10 min on ice and centrifuge again for 5 min. Remove the supernatant and inject an aliquot.

HPLC VARIABLES
Guard column: 75 × 7.5 10 μm SW (TosoHaas, USA)
Column: 600 × 7.5 G2000SW or 600 × 7.5 G3000SW (TosoHaas, USA)
Mobile phase: 25 mM pH 7.0 ammonium bicarbonate
Flow rate: 1
Injection volume: 10-100
Detector: UV 220 or Radioimmunoassay

CHROMATOGRAM
Retention time: 13.93

OTHER SUBSTANCES
Extracted: size isomers

KEY WORDS
comparison with RP-HPLC

REFERENCE
Dalmora,S.; Ezequiel de Oliveira,J.; Affonso,R.; Gimbo,E.; Ribela,M.T.C.P.; Bartolini,P. Analysis of recombinant human growth hormone directly in osmotic shock fluids, *J.Chromatogr.A*, **1997**, *782*, 199–210.

SAMPLE
Matrix: formulations

Sample preparation: Reconstitute somatropin with diluent (Diluent for Humatrope, 5-mL vial, Lilly) to a concentration of 3.33 mg/mL. Dilute 200 μL 3.33 mg/mL solution to 800 μL with highly purified water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 7.5 10 μm G3000SW (TosoHaas, Montgomeryville, PA)
Mobile phase: 10 mM pH 7.3 Sodium phosphate buffer
Flow rate: 0.6
Injection volume: 20
Detector: UV 214

CHROMATOGRAM
Retention time: 12.5-12.8

KEY WORDS
stability-indicating; injections

REFERENCE
Ray,L.R.; Chen,D.A. Stability of somatropin stored in plastic syringes for 28 days, *Am.J.Health-Syst.Pharm.*, **1998**, *55*, 1508–1511.

SAMPLE
Matrix: formulations
Sample preparation: Reconstitute somatropin with diluent (Diluent for Humatrope, 5-mL vial, Lilly) to a concentration of 3.33 mg/mL. Dilute 200 μL 3.33 mg/mL solution to 800 μL with water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Vydac C4
Mobile phase: 1-Propanol:50 mM pH 7.5 Tris buffer 29:71
Column temperature: 45
Flow rate: 0.5
Injection volume: 20
Detector: UV 220

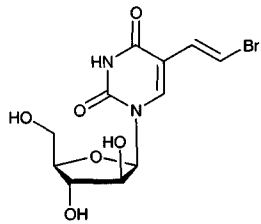
CHROMATOGRAM
Retention time: 31.3-36.3

KEY WORDS
stability-indicating; injections

REFERENCE
Ray,L.R.; Chen,D.A. Stability of somatropin stored in plastic syringes for 28 days, *Am.J.Health-Syst.Pharm.*, **1998**, *55*, 1508–1511.

Sorivudine

Molecular formula: $C_{11}H_{13}BrN_2O_6$
Molecular weight: 349.14
CAS Registry No.: 77181-69-2
Merck Index: 8875



SAMPLE
Matrix: blood

Sample preparation: 250-500 μL Serum + 100 μL 10 μg/mL IS in water, add 5 mL MeCN while mixing, centrifuge. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 1 mL mobile phase, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES

Guard column: 37-53 μm silica gel (Whatman)
Column: 250 × 4.6 5 μm alkyl phenyl (ES Industries)
Mobile phase: MeCN:MeOH:buffer 15:5:80 (Buffer was 50 mM ammonium acetate adjusted to pH 5.0 with glacial acetic acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 295

CHROMATOGRAM

Retention time: 12.5
Internal standard: 1-β-D-arabinofuranosyl-E-5-(2-chlorovinyl)uracil (10)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

protect from light; serum; pharmacokinetics

REFERENCE

Whigan,D.B.; Cohen,A.I. High-performance liquid chromatographic determination of 1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil and its metabolite (E)-5-(2-bromovinyl)uracil in serum, *J.Chromatogr.*, **1991**, *568*, 385–392.

SAMPLE

Matrix: cell suspensions
Sample preparation: Cell suspension + 200 μL ice-cold 4 M perchloric acid, vortex for 1 min, let stand in an ice bath for 5 min, centrifuge at 4° at 2000 g for 10 min. Remove the supernatant and add it to 200 μL 150 μg/mL IS, neutralize with 2 M K_2HPO_4, centrifuge at 4° at 2000 g for 10 min. Free-dry the supernatant, reconstitute in water, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.6 Spherisorb ODS2
Mobile phase: Gradient. MeOH:buffer from 25:75 to 50:50 over 24 min. (Buffer was 50 mM NaH_2PO_4 containing 12.5 mM tetrabutylammonium bromide, adjusted to pH 5 with NaOH. Use a 120 × 4.6 20 μm Partisil column before the injector.)
Column temperature: 48
Flow rate: 1
Injection volume: 10
Detector: UV 292

CHROMATOGRAM

Retention time: 5
Internal standard: E-5-(2-iodovinyl)-2'-deoxyuridine (8)

OTHER SUBSTANCES

Simultaneous: metabolites, phosphorylated species

REFERENCE

Ayisi,N.K.; Wall,R.A.; Wanklin,R.J.; Machida,H.; De Clercq,E.; Sacks,S.L. Comparative metabolism of E-5-(2-bromovinyl)-2'-deoxyuridine and 1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil in herpes simplex virus-infected cells, *Mol.Pharmacol.*, **1987**, *31*, 422–429.

SAMPLE

Matrix: cell suspensions

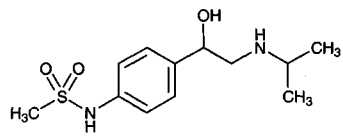
HPLC VARIABLES
Column: Chemopack 300-10C18
Mobile phase: MeOH:10 mM KH_2PO_4 10:50
Flow rate: 1
Detector: UV 290

CHROMATOGRAM
Retention time: 9.64
Limit of detection: 60 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

REFERENCE
Suzuki,S.; Machida,H.; Saneyoshi,M. Antiviral activity of various 1-β-D-arabinofuranosyl-*E*-5-halogenovinyluracils and *E*-5-bromovinyl-2'-deoxyuridine against salmon herpes virus, *Oncorhynchus masou* virus (OMV), *Antiviral Res.*, **1987**, *7*, 79–86.

Sotalol



Molecular formula: $C_{12}H_{20}N_2O_3S$
Molecular weight: 272.37
CAS Registry No.: 3930-20-9, 959-24-0 (HCl)
Merck Index: 8876
Lednicer No.: 1 66; 5 23

SAMPLE
Matrix: blood

HPLC VARIABLES
Column: 100 × 2.1 10 μm Chiralpak AD (Chiral Technologies, Exton, PA)
Mobile phase: EtOH:n-hexane:isopropanol:diethylamine 28.5:63:8.5:0.17
Column temperature: 20
Flow rate: 0.1
Injection volume: 10
Detector: MS, SCIEX API 300 tandem mass, positive ion mode, nebulizer 440°, scan 273.0/213.0

CHROMATOGRAM
Retention time: 5.27, 6.58 (enantiomers)

KEY WORDS
plasma; chiral; small-bore

REFERENCE
Alebic-Kolbah,T.; Zavitsanos,A.P. Chiral bioanalysis by normal phase high-performance liquid chromatography-atmospheric pressure ionization tandem mass spectrometry, *J.Chromatogr.A*, **1997**, *759*, 65–77.

SAMPLE
Matrix: blood
Sample preparation: Condition a Baker 10 C18 SPE cartridge with two 1.5 mL aliquots of MeOH and two 1.5 mL aliquots of 170 mM pH 9.4 N,N-bis(2-hydroxyethyl)glycine buffer. 1 mL Serum + 500 μL 500 mM pH 9.4 N,N-bis(2-hydroxyethyl)glycine buffer + 2 μg IS, vortex, add to the SPE cartridge, wash with two 1.5 mL aliquots of 170 mM pH 9.4 N,N-bis(2-hydroxyethyl)glycine buffer, elute with three 1 mL aliquots of MeCN:ethyl acetate 2:1, freeze the eluate in dry ice/MeOH for 5 s. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm ODS C18 (Altex)
Mobile phase: 10 mM K_2HPO_4 containing 2 mM nonylamine adjusted to pH 2.4 with phosphoric acid (Apply a conditioning injection of 100 μg sotalol and 100 μg IS at the start of the day.)
Column temperature: 40
Flow rate: 2
Injection volume: 100
Detector: UV 235

CHROMATOGRAM
Retention time: 4.5
Internal standard: MJ-6564-1 (Bristol Meyers) (3.4)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, amiodarone, amitriptyline, carbamazepine, desipramine, digoxin, disopyramide, ethosuximide, gentamicin, imipramine, lidocaine, lithium, methotrexate, mexiletine, nortriptyline, phenobarbital, phenytoin, primidone, salicylic acid, theophylline, tobramycin, valproic acid

KEY WORDS
serum; SPE

REFERENCE
Hoyer,G.L. Improved high-performance liquid chromatographic method for the analysis of serum sotalol, *J.Chromatogr.*, **1988**, *427*, 181–187.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 2 μg/mL bisoprolol in water + 200 μL pH 9.2 bicine (N,N-bis(2-hydroxyethyl)glycine) + 4 mL ethyl acetate, shake vigorously for 5 min, centrifuge at 2000 *g* for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL MeOH, inject an aliquot of 100 μL or less.

HPLC VARIABLES
Guard column: 10 mm long LiChrosorb CN
Column: 250 × 4 10 μm LiChrosorb CN
Mobile phase: MeOH:isopropanol:1.16 M perchloric acid 75:25:0.5
Flow rate: 2.5
Injection volume: ≤100
Detector: F ex 235 em 310

CHROMATOGRAM
Retention time: 4.4
Internal standard: bisoprolol (3.6)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Simultaneous: verapamil
Noninterfering: acebutolol, amiodarone, disopyramide, propafenone, hydroquinidine, quinidine

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Poirier,J.-M.; Lebot,M.; Cheymol,G. Rapid and sensitive column liquid chromatographic determination of sotalol in plasma, *J.Chromatogr.*, **1989**, *493*, 409–413.

SAMPLE
Matrix: blood

Sample preparation: 500 μL Serum or plasma + 50 μL 25 μg/mL atenolol in water + 100 μL buffer + 5 mL dichloromethane:isopropanol, rotate at 40 rpm for 5 min, centrifuge at 800 g for 3 min. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen at 55°, reconstitute the residue in 100 μL 50 mM sulfuric acid, vortex for 15 s, inject a 30-50 μL aliquot. (Buffer was prepared by adjusting the pH of a saturated solution of disodium tetraborate to 9 with 6 M HCl.)

HPLC VARIABLES
Column: 150 × 4.5 5 μm ODS Hypersil
Mobile phase: MeCN:10 mM pH 3.2 phosphate buffer 20:80 containing 3 mM sodium 1-octanesulfonate
Flow rate: 1
Injection volume: 30-50
Detector: UV 226

CHROMATOGRAM
Retention time: 5.5
Internal standard: atenolol (4.0)
Limit of detection: 10 ng/mL

KEY WORDS
serum; plasma

REFERENCE
Urech,R.; Chan,L.; Duffy,P. High-performance liquid chromatographic assay of sotalol: improved procedure and investigation of peak broadening, *J.Chromatogr.*, **1990**, *534*, 271–278.

SAMPLE
Matrix: blood
Sample preparation: 300 μL Plasma + 50 μL water:70% perchloric acid 75:25, vortex for 30 s, let stand on ice for 5-10 min, vortex, centrifuge at 3500 g for 5 min, add 30 μL 4 M K_2HPO_4, shake gently by hand, centrifuge at 2500 g for 2 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4 5 μm C18 glass-lined column (Scientific Glass Engineering)
Mobile phase: MeCN:80 mM pH 4.6 KH_2PO_4 6:94
Flow rate: 0.8
Injection volume: 20
Detector: F ex 235 em 310

CHROMATOGRAM
Retention time: 7.1
Limit of quantitation: 80 ng/mL

OTHER SUBSTANCES
Simultaneous: atenolol
Noninterfering: N-acetylprocainamide, disopyramide, flecainide, metoprolol, procainamide, propranolol, quinidine

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Boutagy,J.; Shenfield,G.M. Simplified procedure for the determination of sotalol in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *565*, 523–528.

SAMPLE
Matrix: blood
Sample preparation: Centrifuge plasma at 5000 rpm for 5 min, dilute the supernatant with an equal volume of water, shake vigorously, inject an aliquot onto column A with mobile phase A and elute to waste with mobile phase A, after 10 min backflush the contents of column A onto

column B with mobile phase B, elute with mobile phase B and monitor the effluent. (Two concentration columns (A) are used, one column is being flushed with mobile phase B then mobile phase A while the other is involved in the analysis.)

HPLC VARIABLES
Column: A 40 × 4.6 37-50 μm Bondapak Corasil C18; B 125 × 4.6 5 μm ODS (Shandon)
Mobile phase: A water; B MeCN:10 mM KH_2PO_4 30:70 containing 0.135% dodecylsulfonic acid, adjusted to pH 6.0 with dilute phosphoric acid or KOH
Column temperature: 35
Flow rate: 1
Injection volume: 500
Detector: UV 227

CHROMATOGRAM
Retention time: 10
Limit of quantitation: 10 ng/mL

KEY WORDS
plasma; column-switching; pharmacokinetics

REFERENCE
Herrmann,R. Automated HPLC assay for sotalol in human plasma, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 329–333.

SAMPLE
Matrix: blood
Sample preparation: Evaporate 100 (?) μL 10 μg/mL atenolol in MeOH in to the bottom of a tube, add 500 μL plasma, add 200 μL 1 M pH 9.3 carbonate buffer, add 6 mL ethyl acetate, shake for 4 min, centrifuge at 1000 g for 4 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue with 2 μL 1 M pH 9.3 carbonate buffer and 200 μL 0.005% R-(-)-1-(1-naphthyl)ethyl isocyanate in chloroform (prepared fresh daily), vortex for 15 s, let stand at room temperature for 1 h, evaporate to dryness with a stream of air, add 200 μL MeCN:water 39:61, vortex for 10 s, let stand at room temperature for 1 h, inject a 10 μL aliquot on to column A and column B in series and elute with mobile phase. Column A is subsequently removed from the circuit and backflushed with MeCN:water 50:50 for 3 min, with MeCN for 12 min, with MeCN:water 50:50 for 7.5 min, and with mobile phase for 7.5 min (this is very unclear in the paper).

HPLC VARIABLES
Column: A 75 × 3.9 4 μm Nova-Pak C18; B 100 × 8 4 μm Nova-Pak C18 Radial Compression
Mobile phase: MeCN:water 39:61
Flow rate: 1.5
Injection volume: 10
Detector: F ex 280 em 320

CHROMATOGRAM
Retention time: 23 (+), 26 (-)
Internal standard: atenolol (13)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Noninterfering: metabolites, N-desisopropylpropranolol, 4-hydroxypropranolol, labetalol, 4-methylpropranolol, metoprolol, oxprenolol, pindolol, practolol, propranolol, propranolol glycol, timolol

KEY WORDS
plasma; chiral; pharmacokinetics; derivatization; column-switching

REFERENCE
Hooper,W.D.; Baker,P.V. Enantioselective analysis of sotalol in plasma by reversed-phase high-performance liquid chromatography using diastereomeric derivatives, *J.Chromatogr.B*, **1995**, *672*, 89–96.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 229

CHROMATOGRAM

Retention time: 3.58

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma, whole blood. Condition a Bond-Elut C8 SPE cartridge with MeOH and water. Mix plasma or whole blood, IS, and 50 mM pH 9 borate buffer, add to the SPE cartridge. Wash with water and MeCN. Elute with MeOH. Evaporate the eluate to dryness and reconstitute in 400 μL mobile phase. Inject a 50μL aliquot. Tissue. Homogenize (Braun micro-dismembrator) 100 mg tissue with 100 μL IS solution and 400 μL water while frozen in liquid nitrogen, thaw, rinse twice with 250 μL 1 M pH 3 potassium phosphate buffer. Centrifuge at 2740 g at 20° for 20 min, separate supernatant (S1). Extract pellet with 1 mL MeOH for 15 min with sonication. Centrifuge at 20° for 10 min, evaporate the supernatant to dryness under a stream of nitrogen at 40°. Reconstitute residue in 500 μL 15 mM pH 3 potassium phosphate buffer add to S1, centrifuge. Suck sample slowly through a Bond-Elut C8 SPE cartridge. Wash twice with water, elute twice with 200 μL MeOH, evaporate the eluate to dryness under a stream of nitrogen at 40°. Reconstitute in 500 μL mobile phase. Inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 17 × 4 5 μm Spherisorb C6
Column: 150 × 4.6 5 μm Spherisorb C6
Mobile phase: MeCN:15 mM pH 3 potassium phosphate buffer 17:83 (plasma) or 10:90 (tissue, blood)
Flow rate: 1
Injection volume: 50
Detector: UV 230

CHROMATOGRAM
Retention time: 5.8 (plasma), 7.8 (whole blood, tissue)
Internal standard: atenolol (4.4 (plasma), 6.8 (whole blood, tissue))
Limit of quantitation: 26.5 ng/mL (whole blood, plasma); 270 ng/g tissue

KEY WORDS
whole blood; plasma; heart; SPE

REFERENCE
Laer,S.; Neumann,J.; Scholz,H.; Uebeler,P.; Zimmermann,N. Determination of sotalol in human cardiac tissue by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *681*, 291–298.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 2 mL Plasma + 100 μL 100 μg/mL procainamide in water + 660 μL 2 M perchloric acid, shake briefly, centrifuge at 3000 rpm for 5 min. Remove 1.5 mL of the supernatant and adjust the pH to 9 with 150 μL 4 M NaOH and 4 mL 500 mM boric acid/KCl buffer, add 12 mL water-saturated n-pentanol:chloroform 20:60, shake for 20 min, centrifuge at 3000 rpm for 10 min. Remove the organic phase and dry it over anhydrous sodium sulfate, add 8 mL to 300 μL 100 mM HCl, shake for 10 min, centrifuge at 3000 rpm for 10 min, inject a 25-100 μL aliquot of the aqueous phase. Urine. Dilute urine with pH 9 borate buffer, add 12 mL water-saturated n-pentanol:chloroform 20:60, shake for 20 min, centrifuge at 3000 rpm for 10 min. Remove the organic phase and dry it over anhydrous sodium sulfate, add 8 mL to 300 μL 100 mM HCl, shake for 10 min, centrifuge at 3000 rpm for 10 min, inject a 25-100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeOH:water:acetic acid 38:61:1 containing 1-heptanesulfonic acid (PIC B7)
Flow rate: 1.5
Injection volume: 25-100
Detector: F ex 235 em no filter

CHROMATOGRAM
Retention time: 5.2
Internal standard: procainamide (7)
Limit of detection: 20 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Lefebvre,M.A.; Girault,J.; Saux,M.C.; Fourtillan,J.B. Fluorometric high-performance liquid chromatographic determination of sotalol in biological fluids, *J.Pharm.Sci.*, **1980**, *69*, 1216–1217.

SAMPLE
Matrix: blood, urine

Sample preparation: 1 mL Plasma + 200 μL 2.5 μg/mL S-(-)atenolol + 660 μL 2 M perchloric acid, shake briefly, centrifuge at 2000 g for 5 min. Remove 1 mL of the supernatant and adjust the pH to 9 with 1 mL 2 M Tris-HCl buffer and 250 μL 2 M NaOH, add 5 mL chloroform: isopropanol 75:25, vortex for 1 min, centrifuge at 2000 g for 10 min, repeat the extraction. Combine the organic layers and dry them over about 3 g anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen, reconstitute with 200 μL saturated sodium carbonate solution, add 200 μL 40 μL/mL (-)-menthylchloroformate in MeCN (prepare fresh daily), vortex for 30 s, add 1 mL water, add 2 mL chloroform, vortex for 1 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, centrifuge for 5 min, inject a 40 μL aliquot. Urine. Dilute 1 mL (?) urine 20 times with blank urine, add 200 μL 7.5 μg/mL S-(-)atenolol, adjust the pH to 9 with 1 mL 2 M Tris-HCl buffer, add 5 mL chloroform:isopropanol 75:25, vortex for 1 min, centrifuge at 2000 g for 10 min, repeat the extraction. Combine the organic layers and dry them over about 3 g anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen, reconstitute with 200 μL saturated sodium carbonate solution, add 200 μL 40 μL/mL (-)-menthylchloroformate in MeCN (prepare fresh daily), vortex for 30 s, add 1 mL water, add 2 mL chloroform, vortex for 1 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, centrifuge for 5 min, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm C8 (Jones)
Mobile phase: MeCN:MeOH:water 15:50:35
Flow rate: 2
Injection volume: 40
Detector: F ex 235 em 300

CHROMATOGRAM
Retention time: 14 (l), 15 (d)
Internal standard: S-(-)-atenolol (12.3)
Limit of detection: 12.5 ng/mL
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Simultaneous: alprenolol, captopril, metoprolol, pindolol, propafenone

Noninterfering: acetaminophen, alginic acid, amiloride, amoxicillin, aspirin, diazoxide, digoxin, domperidone, flurazepam, furosemide, glyburide, heparin, hydralazine, hydrochlorothiazide, indapamide, lidocaine,lorazepam, lovastatin, mexiletine, nifedipine, nitroglycerin, omeprazole, oxazepam, procainamide, propranolol, propoxyphene, quinidine, ranitidine, timolol, triamterene, verapamil, warfarin

KEY WORDS
plasma; chiral; derivatization

REFERENCE
Fiset,C.; Philippon,F.; Gilbert,M.; Turgeon,J. Stereoselective high-performance liquid chromatographic assay for the determination of sotalol enantiomers in biological fluids, *J.Chromatogr.*, **1993**, *612*, 231–237.

SAMPLE
Matrix: blood, urine

Sample preparation: Condition a 500 mg Sep-Pak C18 SPE cartridge with 3 mL MeOH and 3 mL 200 mM sodium tetraborate. Dilute urine 10 times with 200 mM sodium tetraborate. Briefly vortex 1 mL plasma with 500 μL saturated pH 9.3 sodium tetraborate. Add 1 mL diluted urine or 1.5 mL diluted plasma to the SPE cartridge, wash with 2 mL 20 mM sodium tetra-

borate, wash with 2 mL water, wash with 1 mL dichloromethane, elute with 5 mL isopropanol. Add 100 μL 100 μg/mL isoamyl p-hydroxybenzoate in MeCN:water 10:90, 100 μL 4 mg/mL 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate in MeCN, and 100 μL 10 mM pH 8.0 $(NH_4)_2HPO_4$ to the eluate, heat at 50° for 3 h, evaporate to dryness under reduced pressure, reconstitute with 200 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: Guard-Pak Resolve C18 (Waters)
Column: 150 × 4.6 5 μm STR ODS II (Shimadzu)
Mobile phase: MeCN:20 mM pH 4.6 $(NH_4)H_2PO_4$ 40:60
Column temperature: 25
Flow rate: 1
Injection volume: 40
Detector: UV 225

CHROMATOGRAM
Retention time: 11.4 ((-)-R), 14.3 ((+)-S)
Internal standard: isoamyl p-hydroxybenzoate (25.5)
Limit of quantitation: 220 ng/mL (urine), 22 ng/mL (plasma)

KEY WORDS
chiral; rat; human; mouse; rabbit; plasma; SPE; derivatization; pharmacokinetics

REFERENCE
Shimizu,T.; Hiraoka,M.; Nakanomyo,H. Enantioselective determination of sotalol enantiomers in biological fluids using high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *674*, 77–83.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.842

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 5 mg amino acids in 10 mL MeCN:water:triethylamine 50:50:0.55. Remove a 50 μL aliquot and add it to 50 μL 0.66% 2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl isothiocyanate (Fluka) in MeCN, shake mechanically for 30 min, add 10 μL 0.26% ethanolamine in MeCN, shake for 10 min, make up to 1 mL with MeCN, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 25 × 4 (sic) 5 μm LiChrospher 100 RP-18
Mobile phase: MeOH:water 80:20
Flow rate: 1
Injection volume: 10
Detector: UV 231

CHROMATOGRAM
Retention time: k' 5.01, k' 6.53 (enantiomers)

OTHER SUBSTANCES
Interfering: atenolol

KEY WORDS
derivatization; chiral

REFERENCE
Lobell,M.; Schneider,M.P. 2,3,4,6-Tetra-O-benzoyl-β-D-glucopyranosyl isothiocyanate: an efficient reagent for the determination of enantiomeric purities of amino acids, β-adrenergic blockers and alkyloxiranes by high-performance liquid chromatography using standard reversed-phase columns, *J.Chromatogr.*, **1993**, *633*, 287–294.

SAMPLE
Matrix: formulations
Sample preparation: Take up in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb C2
Mobile phase: MeCN:buffer 35:65 (1 mL 100 mM HCl + 1200 mL water + 5.84 g NaCl, mix to dissolve, add 700 mL MeOH, make up to 2 L, apparent pH 4.5.)
Flow rate: 1.2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8.2

OTHER SUBSTANCES
Simultaneous: atenolol, nadolol, alprenolol, acebutolol, propranolol, metoprolol, practolol, oxprenolol
Interfering: pindolol, timolol

KEY WORDS
tablets

REFERENCE
Patel,B.R.; Kirschbaum,J.J.; Poet,R.B. High-pressure liquid chromatography of nadolol and other β-adrenergic blocking drugs, *J.Pharm.Sci.*, **1981**, *70*, 336–338.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 500 μL to 10 mL with water. 100 μL Diluted sample + 100 μL 2.5 μg/mL sotalol + 100 μL saturated sodium tetraborate adjusted to pH 9 with HCl + 500 μL

water + 5 mL dichloromethane:isopropanol 3:1, agitate on mechanical shaker for 5 min, centrifuge at 800 g for 3 min. Evaporate organic layer to dryness at 45° under a stream of nitrogen. Dissolve residue in 200 μL 50 mM sulfuric acid, mix for 30 s, inject a 30 μL aliquot.

HPLC VARIABLES
Guard column: Novapak C18 guard insert
Column: 100 × 5 Novapak C18
Mobile phase: MeCN:10 mM potassium phosphate buffer adjusted to pH 3.2 with 0.2 M phosphoric acid 20:80 containing 3 mM 1-octanesulfonic acid
Flow rate: 1
Injection volume: 30
Detector: UV 226

CHROMATOGRAM
Retention time: 5
Internal standard: sotalol

OTHER SUBSTANCES
Simultaneous: atenolol

KEY WORDS
stability indicating; oral liquid; sotalol is IS

REFERENCE
Garner,S.S.; Wiest,D.B.; Reynolds,E.R.,Jr. Stability of atenolol in an extemporaneously compounded oral liquid, *Am.J.Hosp.Pharm.*, **1994**, *51*, 508–511.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.9

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine,

methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 amylose tris(3,5-dimethylphenylcarbamate)
Mobile phase: Hexane:isopropanol:diethylamine 80:20:0.1
Flow rate: 0.5
Detector: UV

CHROMATOGRAM

Retention time: 16 (+), 24 (-)

KEY WORDS

chiral

REFERENCE

Okamoto,Y.; Aburatani,R.; Hatano,K.; Hatada,K. Optical resolution of racemic drugs by chiral HPLC on cellulose and amylose tris(phenylcarbamate) derivatives, *J.Liq.Chromatogr.*, **1988**, *11*, 2147–2163.

SAMPLE

Matrix: solutions
Sample preparation: Evaporate an aliquot of a solution in MeCN containing 625 ng drug to dryness under a stream of nitrogen at room temperature, add 200 μL saturated sodium carbonate, add 200 μL 4% (-)-menthyl chloroformate in MeCN, vortex for 30 s, add an excess amount of 4-hydroxy-L-proline, vortex for 30 s, centrifuge for 3 min, inject a 10-25 μL aliquot of the upper layer.

HPLC VARIABLES

Guard column: 50 × 4.6 Pellicular ODS (Whatman)
Column: 100 × 4.6 5 μm Partisil 5 ODS3
Mobile phase: MeOH:water 60:40
Flow rate: 1
Injection volume: 10-25
Detector: F ex 232 em no emission filter

CHROMATOGRAM

Retention time: 28 (-), 31 (+)

OTHER SUBSTANCES
Simultaneous: metaproterenol

KEY WORDS
derivatization; chiral

REFERENCE
Mehvar,R. Stereospecific liquid chromatographic analysis of racemic adrenergic drugs utilizing precolumn derivatization with (-)-menthyl chloroformate, *J.Chromatogr.*, **1989**, *493*, 402–408.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 200 μM solution in MeOH.

HPLC VARIABLES
Column: 100 × 4.7 7 μm Hypercarb (Shandon)
Mobile phase: MeOH containing 5 mM N-benzyloxycarbonylglycyl-L-proline and 4.5 mM NaOH
Column temperature: 17
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: k' 9.8 (first enantiomer)

KEY WORDS
chiral; α = 1.13

REFERENCE
Huynh,N.-H.; Karlsson,A.; Pettersson,C. Enantiomeric separation of basic drugs using N-benzyloxycarbonyl-glyclyl-L-proline as counter ion in methanol, *J.Chromatogr.A*, **1995**, *705*, 275–287.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 0.71

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 5.96 (A), 3.41 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 33 × 4.6 3 μm Pecosphere CR C18
Mobile phase: MeCN:MeOH:water acidified to pH 2.7 18:2:80 containing 2 g/L sodium octanesulfonate
Flow rate: 4
Injection volume: 10
Detector: UV 238

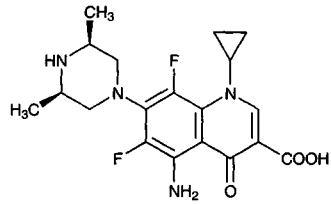
CHROMATOGRAM
Retention time: 0.8

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
high-speed

REFERENCE
Muller,M.C.; Caude,M.; Dauphin,J.F.; Lecointre,L.; Saint-Germain,J. Use of high speed liquid chromatography (HSLC) in the pharmaceutical industry. Practical aspects and limitations, *Chromatographia*, **1995**, *40*, 394–398.

Sparfloxacin



Molecular formula: $C_{19}H_{22}F_2N_4O_3$
Molecular weight: 392.41
CAS Registry No.: 110871-86-8
Merck Index: 8884

SAMPLE
Matrix: aqueous humor, blood, vitreous humor
Sample preparation: Plasma. Acidify plasma with 200 μL 1 M HCl. Filter (Amicon MPS1) while centrifuging at 1000 g for 1 h, inject a 500 μL aliquot of the ultrafiltrate. Vitreous humor. Filter (Amicon MPS1) while centrifuging at 1000 g for 1 h. Acidify ultrafiltrate with one tenth the volume of 330 mM HCl, inject a 200 μL aliquot. Aqueous humor. Filter (Amicon MPS1) while centrifuging at 1000 g for 1 h. Acidify ultrafiltrate with one tenth the volume of 100 mM HCl, inject a 100 μL aliquot

HPLC VARIABLES
Guard column: Corasil C18
Column: μBondapak C18
Mobile phase: MeCN:buffer 13.6:86.4 (Buffer was 25 mM phosphoric acid adjusted to pH 3.0 with tetrabutylammonium hydroxide.)
Flow rate: 1.5
Injection volume: 100-500
Detector: UV 313 (aqueous humor), UV 365 (vitreous humor, plasma)

CHROMATOGRAM
Limit of detection: 10 ng/mL

KEY WORDS
plasma; ultrafiltrate; pharmacokinetics

REFERENCE
Bron,A.M.; Pechinot,A.P.; Garcher,C.P.; Guyonnet,G.A.; Kazmierczak,A.M.; Schott,D.A.; Lecoeur,H. The ocular penetration of oral sparfloxacin in humans, *Am.J.Ophthalmol.*, **1994**, *117*, 322–327.

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL 50 μg/mL IS solution and 2 mL pH 7.5 phosphate buffer to 250 μL plasma. Extract twice with 5 mL portions of ethyl acetate. Evaporate the organic layer. Reconstitute the residue with mobile phase and inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil C8

Mobile phase: MeCN:2 mM phosphoric acid:triethylamine 15:85:0.15, pH 3.0
Flow rate: 1
Detector: UV 308

CHROMATOGRAM
Retention time: 20
Internal standard: ciprofloxacin (10)
Limit of quantitation: 200 ng/mL

KEY WORDS
plasma

REFERENCE
Bhatti,M.M.; Hanson,G.D. Determination of cisapride in human plasma by high-performance liquid chromatography with ultraviolet detection (Abstract 2504), *Pharm.Res.*, **1997**, *14*, S378.

SAMPLE
Matrix: blood
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate..

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:buffer 20:80 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 1
Detector: UV 299

CHROMATOGRAM
Retention time: 8.46
Internal standard: rosoxacin (5.73)

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE
Matrix: blood, CSF, tissue
Sample preparation: Homogenize rat brain in 4 volumes water. 0.05-1 mL Whole blood, serum, CSF or brain homogenate + 1 mL 400 ng/mL IS in 100 mM pH 7.4 phosphate buffer + 5 mL dichloromethane:diethyl ether 80:20, shake for 10 min, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 5 μm YMC Pack A-312 (YMC)
Mobile phase: MeCN:MeOH:5% acetic acid 15:15:70
Column temperature: 40
Flow rate: 1.5
Injection volume: 20
Detector: UV 364

CHROMATOGRAM
Internal standard: 5-amino-7-(3-amino-3-ethyl-1-pyrrolidinyl)-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid
Limit of detection: 5 ng/mL

KEY WORDS
rat; serum; brain; pharmacokinetics

REFERENCE
Naora,K.; Katagiri,Y.; Iwamoto,K.; Tanaka,K.; Yamaguchi,T.; Sekine,Y. Effect of fenbufen on the pharmacokinetics of sparfloxacin in rats, *J.Antimicrob.Chemother.*, **1992**, *30*, 673–683.

SAMPLE
Matrix: blood, CSF, tissue
Sample preparation: Homogenize brain tissue in 20 mM sodium phosphate buffer. Add an equal volume of 4% perchloric acid to homogenate, serum, or CSF, vortex, centrifuge, filter (0.45 μm), add IS, inject an aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:MeOH:5% acetic acid 11:15:74
Flow rate: 3
Injection volume: 100
Detector: UV 364

CHROMATOGRAM
Internal standard: RP41983 (Rhone Poulenc)
Limit of detection: 150 ng/mL

KEY WORDS
brain; serum; pharmacokinetics

REFERENCE
Davey,P.G.; Charter,M.; Kelly,S.; Varma,T.R.K.; Jacobson,I.; Freeman,A.; Precious,E.; Lambert,J. Ciprofloxacin and sparfloxacin penetration into human brain tissue and their activity as antagonists of GABAA receptor of rat vagus nerve, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1356–1362.

SAMPLE
Matrix: blood, feces, urine
Sample preparation: Serum. 500 μL Serum + 100 μL 50 ng/mL ofloxacin in water + 1 mL MeCN, shake mechanically for 30 s, centrifuge at 10000 g for 5 min. Remove 1.5 mL of the supernatant and evaporate it to dryness under reduced pressure at 40°, reconstitute the residue in 300 μL mobile phase, inject a 25 μL aliquot. Urine. Dilute urine with 4 volumes buffer, inject a 50 μL aliquot. Feces. Suspend 100 mg of feces in 9 mL mobile phase, shake for 15 min, centrifuge at 1500 g for 10 min, repeat the extraction twice more, inject a 25 μL aliquot of each extract.

HPLC VARIABLES
Guard column: 30 × 4 30-40 μm Perisorb RP-18 (Merck)
Column: 125 × 4 5 μm Nucleosil 100 SA
Mobile phase: MeCN:buffer 75:25 adjusted to pH 3.82 with concentrated phosphoric acid, final sodium concentration 23 mM (Buffer was 6.74 mL concentrated phosphoric acid and 40 mL 2 M NaOH made up to 990 mL with water, adjust pH to 2.92 with concentrated phosphoric acid, make up to 1 L with water.)
Flow rate: 1.5
Injection volume: 25-50
Detector: F ex 295 em 525

CHROMATOGRAM
Retention time: 4.7
Internal standard: ofloxacin (8.0)
Limit of detection: 140 ng/mL (feces), 460 ng/mL (urine), 50 ng/mL (serum)

KEY WORDS
serum; protect from light

REFERENCE

Borner,K.; Borner,E.; Lode,H. Determination of sparfloxacin in serum and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *579*, 285–289.

SAMPLE

Matrix: blood, intestinal efflux

Sample preparation: Intestinal efflux. Freeze intestinal efflux at -80°, lyophilize, reconstitute with 1 mL ofloxacin in MeOH:100 mM phosphoric acid 50:50, centrifuge at 3000 rpm for 10 min, inject a 20 μL aliquot. Serum. Deproteinize serum with MeOH containing ofloxacin, extract with dichloromethane at pH 7.5.

HPLC VARIABLES

Column: 150 × 3.9 Novapack C18

Mobile phase: MeOH:buffer 35:65 (Buffer was 10 mM pH 3.0 potassium phosphate buffer containing 25 mM sodium heptanesulfonate (PIC B7) and 20 mM triethylamine.)

Flow rate: 1.5

Injection volume: 20

Detector: UV 364

CHROMATOGRAM

Internal standard: ofloxacin

Limit of detection: 100 ng/mL

KEY WORDS

serum; rat

REFERENCE

Rubinstein,E.; Dautrey,S.; Farinoti,R.; St.Julien,L.; Ramon,J.; Carbon,C. Intestinal elimination of sparfloxacin, fleroxacin, and ciprofloxacin in rats, *Antimicrob.Agents Chemother.*, **1995**, *39*, 99–102.

SAMPLE

Matrix: blood, tissue

Sample preparation: Homogenize (Ultra-Turrax T25) mouse lung in 1-3 mL pH 6.8 Soerensen phosphate buffer, centrifuge. Extract serum or lung homogenate supernatant, extract using a Bond Elut C2 SPE cartridge, inject a 100 μL aliquot of the extract.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Ultrabase C8 (SFCC, Neuilly Plaisance, France)

Mobile phase: MeCN:MeOH:5% acetic acid 15:10:75

Flow rate: 1

Injection volume: 100

Detector: UV 280

CHROMATOGRAM

Retention time: 6

Internal standard: sparfloxacin

Limit of detection: 15 ng

OTHER SUBSTANCES

Extracted: temafloxacin

KEY WORDS

serum; lung; mouse; pharmacokinetics; SPE; sparfloxacin is IS

REFERENCE

Vallée,E.; Azoulay-Dupuis,E.; Bauchet,J.; Pocidalo,J.-J. Kinetic disposition of temafloxacin and ciprofloxacin in a murine model of pneumococcal pneumonia. Relevance for drug efficacy, *J.Pharmacol.Exp.Ther.*, **1992**, *262*, 1203–1208.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. Condition an AASP SPE cartridge (Varian) with MeOH. Centrifuge plasma at 1000 g for 10 min. 500 μL Plasma + 50 μL 20 μg/mL IS, add to the SPE cartridge, wash with water, elute the contents of the SPE cartridge onto the analytical column with mobile phase. Urine. Add internal standard, inject an aliquot directly. Hydrolyze conjugates with 1 M NaOH for 10 min.

HPLC VARIABLES

Column: 150 × 6 Asahi PAK OD1,50 reverse phase
Mobile phase: 5% methanol-acetonitrile-acetic acid (11.6/11.6/76.8) (sic) [Perhaps MeCN:MeOH: 5% acetic acid 11.6:11.6:76.8]
Detector: UV 364

CHROMATOGRAM

Internal standard: 1-ethyl-6-chloro-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid
Limit of quantitation: 250 ng/mL (urine), 15 ng/mL (plasma)

KEY WORDS

plasma; SPE; pharmacokinetics

REFERENCE

Fillastre,J.P.; Montay,G.; Bruno,R.; Etienne,I.; Dhib,M.; Vivier,N.; Le Roux,Y.; Guimart,C.; Gay,G.; Schott,D. Pharmacokinetics of sparfloxacin in patients with renal impairment, *Antimicrob.Agents Chemother.*, **1994**, *38*, 733–737.

SAMPLE

Matrix: growth medium
Sample preparation: 500 μL Sample + 500 μL 100 μg/mL IS in cold (4°) MeCN, vortex, centrifuge at 3000 g for 5 min. Remove a 500 μL aliquot of the supernatant, filter (0.45 μm Acrodisc syringe filter), inject a 30 μL aliquot. (Protect all specimens from light.)

HPLC VARIABLES

Guard column: C18 5U (Alltech)
Column: 150 × 4.6 7 μm Adsorbosphere HS C18 7U
Mobile phase: MeCN:20 mM pH 3.0 phosphate buffer 35:65 containing 0.2% triethylamine and 0.2% sodium dodecyl sulfate, adjusted to pH 3.0 with 85% phosphoric acid
Flow rate: 1.75
Injection volume: 30
Detector: UV 280

CHROMATOGRAM

Retention time: 7.09
Internal standard: ciprofloxacin (4.67)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Also analyzed: ciprofloxacin, clinafloxacin, levofloxacin, ofloxacin, temafloxacin, trovafloxacin

KEY WORDS

Mueller-Hinton broth

REFERENCE

Wright,D.H.; Herman,V.K.; Konstantinides,F.N.; Rotschafer,J.C. Determination of quinolone antibiotics in growth media by reversed-phase high-performance liquid chromatography, *J.Chromatogr.B*, **1998**, *709*, 97–104.

SAMPLE

Matrix: microsomal incubations
Sample preparation: Centrifuge 250 μL microsomal incubation at 13000 g for 5 min, neutralize perchloric acid lysates with 1 M potassium carbonate, add IS, inject an aliquot directly.

HPLC VARIABLES
Column: Asahipack ODP 50
Mobile phase: Gradient. MeCN:water from 10:90 to 40:60 over 10 min
Detector: UV

CHROMATOGRAM
Internal standard: ciprofloxacin

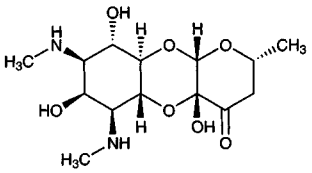
REFERENCE
Rispal,P.; Grellet,J.; Celerier,C.; Breilh,D.; Dorian,M.; Pellegrin,J.L.; Saux,M.C.; Leng,B. Comparative uptake of sparfloxacin and ciprofloxacin into human THP 1 monocytic cells, *Arzneimittelforschung*, **1996**, *46*, 316–319.

SAMPLE
Matrix: solution

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 35:65 containing 300 mM KCl
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 291

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

Spectinomycin



Molecular formula: $C_{14}H_{24}N_2O_7$
Molecular weight: 332.35
CAS Registry No.: 1695-77-8, 22189-32-8 (di HCl pentahydreate), 21736-83-4 (di HCl)
Merck Index: 8890

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 400 μL MeCN:trifluoroacetic acid 97:3, vortex, centrifuge at 2000 g for 3 min. Remove a 250 μL aliquot of the supernatant and add it to 200 μL 5 mg/mL 2,4-dinitrophenylhydrazine in MeCN, mix, heat at 70° for 1 h, cool on ice for 2 min, add 30 μL acetone, mix, heat at 70° for 10 min, cool on ice, filter (Ultrafree MC 30000 molecular weight limit) while centrifuging at 2000 g, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 33 × 4.6 3 μm Pecosphere C18 CR
Mobile phase: Gradient. A was MeCN:water 60:40. B was MeCN:MeOH 19:1. A:B 100:0 for 1 min, to 0:100 over 10 min, maintain at 0:100 for 2 min, re-equilibrate at initial conditions for 10 min.
Flow rate: 1.2
Injection volume: 20
Detector: UV 405

CHROMATOGRAM
Retention time: 9
Limit of quantitation: 2 μg/mL

KEY WORDS
plasma; turkey; derivatization

REFERENCE
Burton,S.D.; Hutchins,J.E.; Fredericksen,T.L.; Ricks,C.; Tyczkowski,J.K. High-performance liquid chromatographic method for the determination of spectinomycin in turkey plasma, *J.Chromatogr.*, **1991**, *571*, 209–216.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL C18 SPE cartridge (J.T. Baker) with 3 mL MeOH, 2 mL 20 mM sodium dioctylsulfosuccinate (slowly!), and 3 mL 20 mM pH 5.6 citric acid, do not allow to go dry. 2 mL Plasma + 8 mL water + 625 μL MeOH, adjust pH to 5.2-5.7 with about 60 μL 1 M HCl, vortex for 30 s, centrifuge at 2500 g for 10 min, remove the supernatant, resuspend the solid in 3 mL 20 mM pH 5.6 citric acid, centrifuge. Combine the supernatants and add them to the SPE cartridge at 1-2 mL/min, wash with two 3 mL portions of 20 mM pH 5.6 citric acid, allow to go dry. Centrifuge the SPE cartridge at 3000 g for 15 min, dry with a stream of nitrogen for 20 min, elute with 3 mL MeOH at 2 drops/s. Evaporate the eluate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 2 mL 10 g/L sodium bicarbonate containing 100 μL/L 1-methylpyrrole, vortex for 30 s, add 3 mL 12 mg/mL 2-naphthalenesulfonyl chloride in MeCN (prepare just before derivatization), vortex for 30 s, heat at 100° for 15 min, cool to room temperature, add 1 mL n-butyl chloride, vortex twice for 20 s, centrifuge at 1500 g for 5 min. Remove 2 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 1 mL n-butyl chloride, vortex for 30 s, centrifuge at 2500 g for 10 min, inject a 50 μL aliquot onto column A and elute to waste with mobile phase A, after 8 min divert the effluent from column A containing spectinomycin onto column B, after 2 min remove column A form the circuit, elute column B with mobile phase B, monitor the effluent from column B. Backflush column A with mobile phase A for 15 min.

HPLC VARIABLES
Column: A 10 × 2.1 40 μm pellicular silica + 100 × 3 5 μm Chromspher silica glass cartridge (Chrompack); B two 100 × 3 5 μm Chromspher silica glass cartridges in series (Chrompack)
Mobile phase: A Dichloromethane:MeCN:ethyl acetate:acetic acid 75:7.5:1.8:0.425; B Dichloromethane:MeCN:ethyl acetate:acetic acid 100:20:3.6:0.85
Flow rate: A 0.4; B 0.6
Injection volume: 50
Detector: UV 250

CHROMATOGRAM
Retention time: 14
Limit of quantitation: 40 ng/mL

KEY WORDS
derivatization; cow; calf; chicken; pig; plasma; SPE; use glass not plastic tubes; column-switching; heart-cut; normal phase

REFERENCE
Haagsma,N.; Keegstra,J.R.; Scherpenisse,P. High-performance liquid chromatographic determination of spectinomycin in swine, calf and chicken plasma, *J.Chromatogr.*, **1993**, *615*, 289–295.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL High-hydrophobic C18 SPE cartridge (J.T. Baker) with 3 mL MeOH, 2 mL 20 mM sodium dioctyl sulfosuccinate (homogenize before use, pass slowly through cartridge), and 4 mL 20 mM pH 5.6 citric acid buffer, do not allow to go dry. 2 mL Plasma + 8 mL water + 625 μL MeOH, adjust pH to 5.2-5.7 with ca. 60 μL 1 M HCl, vortex for 30 s, centrifuge at 2500 g for 10 min, remove the supernatant, rinse the residue with 3 mL citric acid buffer, centrifuge. Combine the rinse and the supernatant and add them to the SPE cartridge at 1-2 mL/min, wash with two 3 mL portions of citric acid buffer, allow to run dry. Centrifuge the SPE cartridge at 3000 g for 20 min then dry it in a stream of nitrogen for 20 min, elute with 4 mL MeOH, evaporate the eluate to dryness under a stream of nitrogen at

room temperature, reconstitute with 1 mL MeOH:water 20:80, vortex, centrifuge at 2500 g for 10 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 10 × 4.6 cation-exchange (Phase-Sep)
Column: 250 × 4.6 5 μm Spherisorb SCX
Mobile phase: MeCN:100 mM pH 2.6 sodium sulfate 20:80
Flow rate: 1.5
Injection volume: 50
Detector: F ex 340 em 460 following post-column reaction. The column effluent mixed with reagent A pumped at 0.25 mL/min and reagent B pumped at 0.75 mL/min and the mixture flowed through a 2.2 m × 0.5 mm i.d. coil at 70°. The effluent from the coil was mixed with reagent C pumped at 1 mL/min and this mixture flowed through a 1.7 m × 0.3 mm i.d. PTFE coil at 25° to the detector. Reagent A was prepared by adding 10 mL sodium hypochlorite solution (13% active chlorine) and 10 mL 100 mM pH 7.0 potassium phosphate buffer to 980 mL water. Reagent B was 400 mM pH 10.2 boric acid buffer prepared by dissolving 24.4 g boric acid and 20.0 g KOH in 1 L water. Reagent C was prepared by adding 10 mL 80 mg/mL o-phthaldialdehyde in EtOH and 1 mL 2-mercaptoethanol to 990 mL 300 mM pH 10.2 boric acid buffer. (The 300 mM pH 10.2 boric acid buffer was prepared by dissolving 18.55 g boric acid and 15.0 g KOH in 1 L water.)

CHROMATOGRAM

Retention time: 9
Limit of quantitation: 60 ng/mL

KEY WORDS

pig; cow; chicken; plasma; SPE; post-column reaction

REFERENCE

Haagsma,N.; Scherpenisse,P.; Simmonds,R.J.; Wood,S.A.; Rees,S.A. High-performance liquid chromatographic determination of spectinomycin in swine, calf and chicken plasma using post-column derivatization, *J.Chromatogr.B*, **1995**, *672*, 165–171.

SAMPLE

Matrix: bulk
Sample preparation: 10 mL 250 μg/mL Spectinomycin solution in buffer + 10 mL 10 mg/mL 2-naphthalenesulfonyl chloride in MeCN containing 200 μg/mL methylprednisolone acetate (prepare fresh daily), shake, heat at 100° for 10 min, cool to room temperature, make up to 50 mL with mobile phase, shake vigorously for 10 min, centrifuge at <300 g for 3-5 min, inject a 40 μL aliquot of the organic layer. (Buffer was 4.2 g/L sodium bicarbonate containing 100 μL/L 1-methylpyrrole.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm LiChrosorb SI-60
Mobile phase: Butyl chloride:THF:ethyl acetate:isopropanol:acetic acid 86:3.7:3:2.5:5 (Butyl chloride was 50% water-saturated.)
Flow rate: 1
Injection volume: 40
Detector: UV 254

CHROMATOGRAM

Retention time: 10
Internal standard: methylprednisolone acetate (18)
Limit of detection: 4.2 ng

OTHER SUBSTANCES

Simultaneous: actinosapectinoic acid

KEY WORDS

derivatization; normal phase

REFERENCE
Tsuji,K.; Jenkins,K.M. Derivatization of secondary amines with 2-naphthalene-sulfonyl chloride for high-performance liquid chromatographic analysis of spectinomycin, *J.Chromatogr.*, **1985**, *333*, 365–380.

SAMPLE
Matrix: solutions
Sample preparation: Prepare an aqueous solution, inject an aliquot.

HPLC VARIABLES
Guard column: 43 × 4.2 Perisorb RP-8 (Merck)
Column: 250 × 4.6 LiChrosorb RP-8
Mobile phase: 0.1% Acetic acid containing 20 mM sodium heptanesulfonate and 200 mM sodium sulfate
Flow rate: 2
Injection volume: 15
Detector: F ex 350 em 450 following post-column reaction. The column effluent mixed with 10 mM sodium hypochlorite in 400 mM pH 10.4 potassium borate buffer pumped at 0.5 mL/min and the mixture flowed through a 2 m × 0.5 mm i.d. PTFE coil at 100° and was mixed with the reagent pumped at 0.5 mL/min. This mixture passed through a 2 m × 0.5 mm i.d. PTFE coil at ambient temperature to the detector. (The reagent was prepared by adding 10 mL 80 mg/mL o-phthalaldehyde in 95% EtOH to 1 L 400 mM boric acid solution containing 2 mL 2-mercaptoethanol adjusted to pH 9.7 with KOH, then adding 1 g/L Brij. (Proc. Nat. Acad. Sci. USA 1975, 72, 619).)

CHROMATOGRAM
Retention time: 7.5

OTHER SUBSTANCES
Simultaneous: actinamine, actinospectinoic acid, dihydrospectinomycin

KEY WORDS
post-column reaction

REFERENCE
Myers,H.N.; Rindler,J.V. Determination of spectinomycin by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1979**, *176*, 103–108.

SAMPLE
Matrix: solutions
Sample preparation: Inject 50 μL of a solution in 150 mM sodium acetate.

HPLC VARIABLES
Guard column: 25 × 4 IonPac CG3 (Dionex)
Column: 250 × 4 IonPac CS3 (Dionex)
Mobile phase: 150 mM pH 6 sodium acetate buffer
Column temperature: 21
Flow rate: 1
Injection volume: 50
Detector: E following post-column reaction, Dionex pulsed-amperometric detector, gold working electrode, stainless steel auxiliary electrode, Ag/AgCl reference electrode, rise-time filter 3.0 s, output range 1 μA. Pulse sequence was +0.05 V sampling potential with 100 ms delay-time and 380 ms measuring time followed by +0.60 V oxidation potential to clean the electrode for 120 ms and -0.70 V reduction potential to activate the electrode for 60 ms. The column effluent mixed with 100 mM NaOH pumped at 0.5 mL/min and the mixture flowed to the detector.

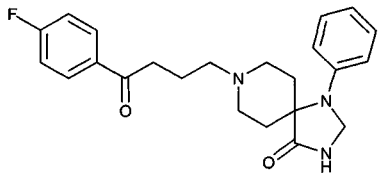
CHROMATOGRAM
Retention time: 10.7
Limit of detection: 20 ng
Limit of quantitation: 60 ng

OTHER SUBSTANCES
Simultaneous: actinamine, actinospecinoic acid

KEY WORDS
post-column reaction

REFERENCE
Phillips,J.G.; Simmonds,C. Determination of spectinomycin using cation-exchange chromatography with pulsed amperometric detection, *J.Chromatogr.A*, **1994**, *675*, 123–128.

Spiperone



Molecular formula: $C_{23}H_{26}FN_3O_2$
Molecular weight: 395.48
CAS Registry No.: 749-02-0
Merck Index: 8903

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 500 μL 500 mM pH 8.5 phosphate buffer, extract with 2.5 mL heptane:isoamyl alcohol 98:2. Shake for 5 min, centrifuge at 1700 g for 10 min. Mix 2.0 mL organic layer with 100 μL 3 M acetic acid, shake for 20 min, centrifuge at 1700 g for 10 min. Aspirate organic layer, inject a 20 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 150 × 4.6 5 μm EicomPak MA-5ODS (Eicom, Japan)
Mobile phase: MeCN:MeOH:buffer 20:15:65 containing 500 μg/L disodium EDTA (Buffer was 100 mM KH_2PO_4 adjusted to pH 3.5 with 100 mM phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: E, Eicom ECD-100, glassy carbon electrode +1 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 14.9
Internal standard: spiperone

KEY WORDS
plasma; rat; spiperone is IS

REFERENCE
Takayasu,T.; Kakubari,I.; Fukamachi,A.; Mafune,E.; Takasugi,N.; Takayama,K.; Nagai,T. Determination of timiperone in rat plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.B*, **1996**, *679*, 161–165.